Electronic Supplementary Information (ESI)

Enhanced synergism of thermo-chemotherapy by combining highly efficient magnetic hyperthermia with magnetothermally-facilitated drug release

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Experimental procedures:

Synthesis of thermosensitive polymer PLA-*b*-poly(N-*co*-D):

Following the relevant literatures,[1-4] the PLA-*b*-poly(N-*co*-D) was synthesized by ring opening polymerization (ROP) and reversible addition fragmentation chain transfer (RAFT) polymerization, as shown in Fig. S1. The details are shown in below.

First of all, the S-1-Dodecyl-S′-(α,α′-dimethyl-α″-acetic acid)trithiocarbonate (DDAT) was selected as chain transfer agent (CTA) for RAFT polymerization and synthesized according to literature.[1]

Synthesis of PLA: PLA was synthesized by ROP using 1-Pyrenemethanol (Py-OH) as a initiator and $Sn(Oct)_2$ as a catalyst. The molar ratio of Py-OH/D,L-lactide was 1/30. The whole reaction system was placed in oil bath at 115 ^oC for 24 h under nitrogen atmosphere with magnetic stirring. After the reaction, the resulting product was dissolved in dichloromethane (CH_2Cl_2) and precipitated three times with cold diethyl ether. The purified PLA was dried in a vacuum oven at room temperature until constant weight.

Preparation of macro-CTA (PLA-DDAT) for RAFT polymerization: The reaction was carried out by Steglich esterification, according to established method.[2] PLA, DDAT, dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)-pyridin (DMAP) were mixed and dissolved in CH_2Cl_2 with corresponding feed

molar ratios as 1/3/3/1. The whole reaction proceeded in nitrogen and ambient temperature for 48 h. After the reaction, the solution was filtered and concentrated. The resulting product was precipitated in cold ether. Then the precipitate was purified by reprecipitation in excess amount of cold ether other four times. At last, the purified PLA-DDAT was dried by freeze-dried and preserved in low temperature.

Synthesis of thermosensitive copolymer PLA-*b*-poly(N-*co*-D): The PLA-*b*-poly(N-*co*-D) was synthesized by RAFT polymerization.[3,4] All reagents including *N*-isopropylacrylamide (NIPAM), *N,N*-dimethylacrylamide (DMAM), PLA-DDAT were dissolved in dioxane, then added *N,N*′-azobisisobutyronitrile (AIBN) as a catalyst and mixed completely. In order to adjust the LCST of PLA-*b*-poly(N-*co*-D), the feed molar ratio of NIPAM and DMAM varied as 5/2.0, 5/2.2 and 5/2.4. Those reactions were carried out at 70 ^{o}C for 6 h under nitrogen atmosphere. After polymerization, products were precipitated in excess diethyl ether. Then the precipitates were purified by reprecipitation in excess amount of cold ether other two times. At last, those precipitates were dried by freeze-dried and preserved in low temperature.

Characterization of polymer composition and structure:

^{1}H NMR spectra were obtained from a Bruker DMX 500 NMR spectrometer with $CDCl_3$ as the solvent. The chemical shifts were relative to tetramethylsilane. The molecular weight and molecular-weight distribution were measured on a Viscotek 270 gel-permeation chromatography instrument. Dimethylformamide (DMF) was used as the eluent at a flow rate of 1.0 mL min^{-1} at 35 °C. The molecular weights were calculated against polystyrene standards.

Synthesis and characterization of hydrophobic monodispersed magnetic nanoparticles $Mn_{0.6}Zn_{0.4}Fe_2O_4$ (MZF):

The MZF was synthesized by established procedure.[5] Briefly, $Fe(acac)_3$ (2 mmol) and certain $Mn(acac)_2$ and $Zn(acac)_2$ with corresponding molar ratios as 10/3/2 were mixed with 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), and oleylamine (6 mmol) in benzyl ether (20 mL) under dry and deoxidized argon atmosphere. The mixture was heated to 200 °C for 2 h and then heated to reflux (≈300 °C) for another hour. After cooling to room temperature, the solution was treated with ethanol and then centrifuged to yield a dark-brown precipitate. The MZF was redispersed in hexane and reprecipitated with ethanol. Finally, these magnetic nanoparticles were dispersed in anhydrous hexane for storage.

The structural formations of $Mn_xZn_{1-x}Fe_2O_4$ were characterized by XRD with Rigaku D/Max-2550VB3+ (Cu-Kα radiation, 40 kV, 100 mA, Japan). The morphology of $Mn_xZn_{1-x}Fe_2O_4$ was characterized by HRTEM (JEOL JEM-2010F, 200 KV, Japan).

Preparation of thermo-responsive nanocarrier (TRN) and magnetothermally-responsive nanocarrier (MTRN):

All nanocarriers and micelles were prepared by one-step self-assembly. For TRNs, PLA-*b*-poly(N-*co*-D) was dissolved in THF completely with the concentration of 2 mg mL^{-1}. The mixed solution was then slowly added into deionized water with sonication. The mixture was dialyzed over night to remove THF. The MTRNs were prepared as follows. The PLA-*b*-poly(N-*co*-D) and MZF were mixed and dissolved in THF with mass ratio of 1/1. The solution was subsequently added into deionized water with sonication and dialyzed over night to remove THF. The CPT loaded MTRN (CPT/MTRN) was prepared by same method with feed ratio of PLA-*b*-poly(N-*co*-D), MZF and CPT as 45/45/10. At last, we also prepared CPT loaded thermo-responsive nanocarrier (CPT/TRN) by the same procedure with mass ratio of CPT/ PLA-*b*-poly(N-*co*-D) as 1/9. The Nile red loaded MTRN (NR/MTRN) was prepared by same method. All products were freeze-dried and preserved in low temperature. It is worth noting that all kinds of nanocarriers were prepared and preserved in dark.

Magnetocaloric Effect of CPT/MTRN:

The magnetocaloric effect of CPT/MTRN was performed by the alternating magnetic field (AMF) generator (SPG-20AB, ShuangPing tech. Ltd., China). The frequence (*f*) of the AMF was fixed at 114 kHz, and the strength of AMF ($H_{applied}$) was adjusted ranging from 0−115.1 kA m^{-1}. CPT/MTRNs were dispersed into deionized water to form colloids with 0.1 mg mL^{-1} of MZFs, in which the concentration of MZFs was quantified by inductively coupled plasma atomic emission spectroscopy (ICP-AES). In all measurements, 2 mL colloidal solutions of nanocomposite micelles were exposed to different oscillating AMF (62.8 kA m^{-1}, 89.9 kA m^{-1}, 114.6 kA m^{-1}) with the same *f* (114 kHz) in the center of the copper coil (inner diameter of 18 mm). The temperature changes were recorded using a computer-attached fiber optic temperature sensor (FOT-M, FISO, Canada). The specific adsorption rate (SAR) of each sample was calculated from Equation (1) [6]:

$$SAR = C\frac{\Delta T}{\Delta t} * \frac{1}{m_{Fe} + m_{Mn} + m_{Zn}} \quad (1)$$

In Equation 1, C is the specific heat of deionized water (C_{water}= 4.18 J g^{-1} K^{-1}), $\Delta T/\Delta t$ is the initial slope of the time-dependent temperature curve at the first 20 s, and the $m_{Fe} + m_{Mn} + m_{Zn}$ is the weight fraction of the metal element in the sample.

Drug release behavior of CPT/MTRNs under different condition:

The magnetothermally-response of CPT/MTRN on its drug release was investigated by the AMF generator with f of 114 kHz and $H_{applied}$ of 89.9 kA m^{-1}. Meanwhile, the thermo-responsive related drug release behaviors were studied at different temperatures (43 ^{o}C and 37 ^{o}C) as control.

Without AMF, 2 mL CPT/MTRN solution (PBS, 2 mg mL^{-1}) was dialyzed against 38 mL PBS at different temperatures (43 ^{o}C and 37 ^{o}C) for 24 h. Under an external AMF, magnetothermally-responsive drug release was performed by a similar procedure for 15 min. At predetermined time intervals, 0.5 mL solution was extracted from a dialysis bag outside and replaced with fresh PBS. CPT content in the extracted solution was measured by UV/Vis spectrophotometry and the cumulative release was calculated.

Cell Culture:

SK-OV-3 and HepG2 cell lines were provided by the Institute of Biomedical Engineering & Nano Science, Tongji University (China). SK-OV-3 was cultured in McCOY's 5A (Gibco) containing 10% fetal bovine serum (FBS, Hyclone). HepG2 was cultured in DMEM (high glucose, Gibco) containing 10% FBS. These cell lines were cultured in incubator at 37 ^{o}C, 5% CO_2 and humidified circumstance.

Biocompatibility of MTRNs:

In vitro biocompatibility of MTRNs was assessed by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT). Two types of cells were incubated with MTRNs in 96-well plate, in which the concentration of the samples varied from 100 μg mL^{-1} to 1000 μg mL^{-1}. After incubating for 72 h, cell viability was investigated by the standard MTT method.[7] Meanwhile the culture medium was selected as control and each sample was reduplicated five times. The cell survival rate was calculated as the percentage of control values.

Anti-tumor efficiency of thermo-chemotherapy by CPT/MTRN under AMF:

For efficiency research, cells were incubated in culture dish (35 mm). Anti-tumor efficiencies of CPT/MTRN under different condition were evaluated by MTT, in which the CPT concentrations varied from 0 μg mL^{-1} to 20 μg mL^{-1} and the concentration of MZF fixed at 100 μg mL^{-1}. Two types of cells were incubated with CPT/MTRNs under non-AMF or AMF (5 min per 24 h), respectively. After exposure to AMF, the cells were cultured in incubator for 24 h. After incubating, cell survival was assessed by MTT quantitatively. Each sample was reduplicated four times. Meanwhile, cytotoxicity of free CPT (low concentration DMSO as hydrotropy agent) was carried out as positive control and pure culture medium (containing 10% FBS and 1% PS) as negative control. The relative cell survival was calculated as the percentage of negative control values.

Cellular uptake experiments of drug by drug-loaded MTRN under different condition:

The cellular uptake experiments were performed by flow cytometer (FCM) and confocal laser scanning microscopy (CLSM). Nile red was used as a hydrophobic fluorescence probe. And cells were incubated in culture dish (35 mm).

For FCM, two types of cells were incubated with NR/TMRNs without AMF for 5 min, 60 min and 240 min as NR/MTRN group. In comparison, cells were incubated with NR/MTRNs under AMF for 5 min. Then the cells were cultured back to incubator for prolonged 0 min, 55 min and 235 min as NR/MTRN+MH group. After that, all cells of each sample were collected and treated by standard procedure described in relevant literature.[8] Similarly, cells incubated with culture medium were selected as control.

For CLSM, two types of cells were treated by same procedures called NR/MTRN group or NR/MTRN+MH group. After that, all cells of each sample were treated by standard procedure described in relevant literature.[9]

Quantitative analysis of Heat Shock Protein (HSP70) expression:

The HSP70 expression was quantified by enzyme-linked immunosorbent assay (ELISA). The two types of cells were seeded in culture dish (35 mm) and incubated with pure MTRNs, MTRNs added period magnetic hyperthermia (MTRN+MH, 5 min per 24 h), CPT/MTRNs with non-AMF or AMF (CPT/MTRN+MH, 5 min per 24 h) respectively. And culture times were fixed as 24 h, 48 h and 72 h. Each

sample was reduplicated three times. At the end of the prescribed time periods, all cells of each sample were lysed by lysis buffer (Beyotime, China) and assayed by BCA kit (Pierce) and ELISA kit of HSP70 (Abcam). The standard procedures followed the introduction books of those kits. At the same time, cells incubated with pure culture medium were selected as negative control. The relative HSP70 expressions by different treatments were calculated as the percentage of negative control values.

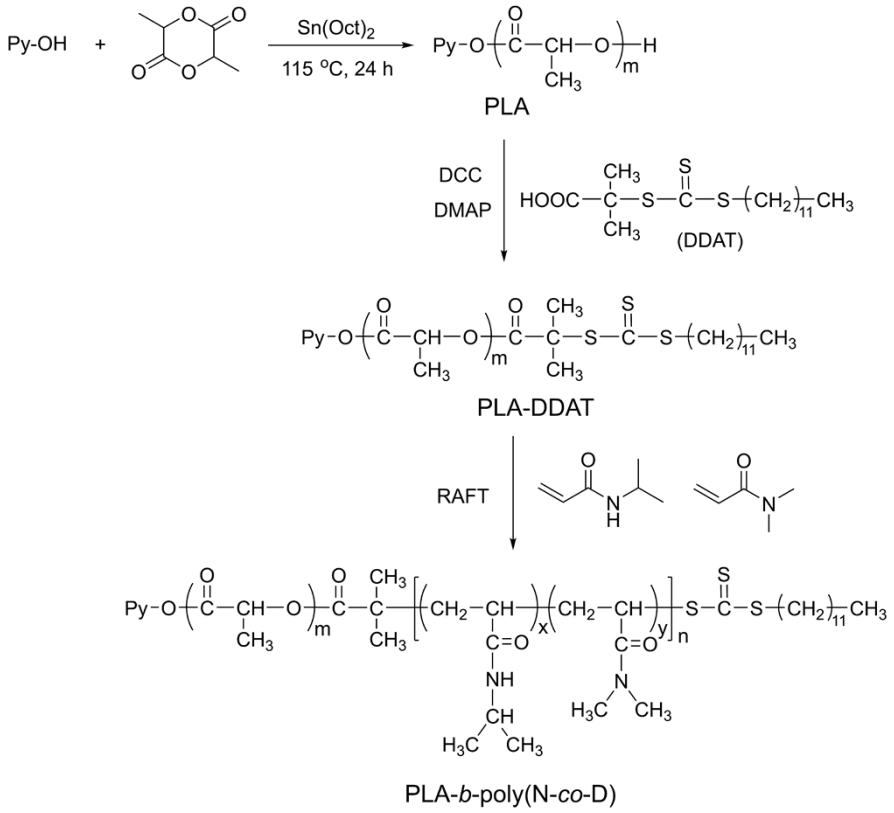

Fig. S1. Synthetic scheme of amphiphilic thermosensitive copolymer PLA-*b*-poly(N-*co*-D) by combination of ROP and RAFT polymerization.

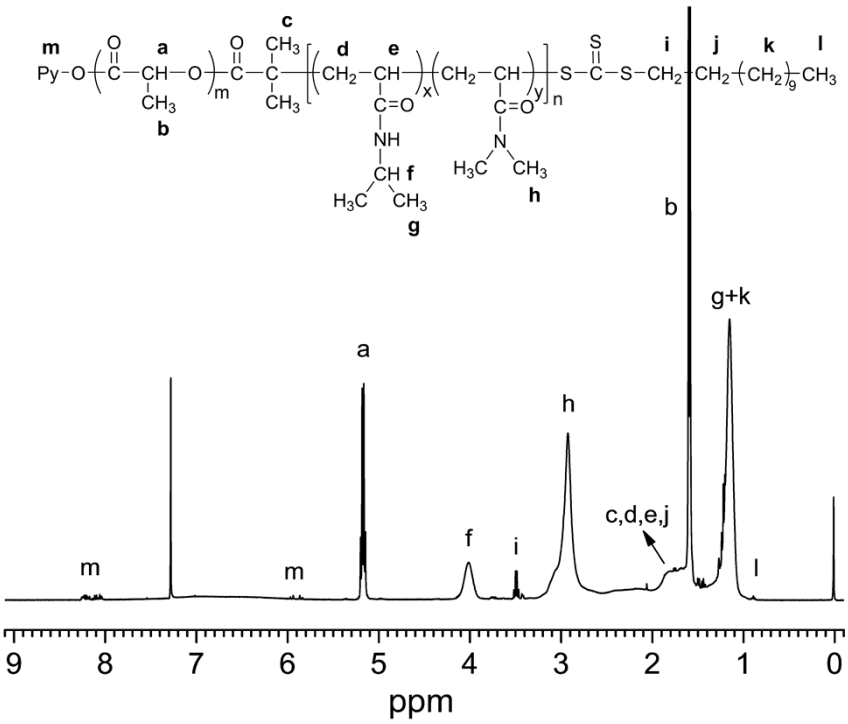

Fig. S2. ^{1}H NMR spectrum of PLA-*b*-poly(N-*co*-D), marking all characteristic proton peaks based on relevant references[3,4].

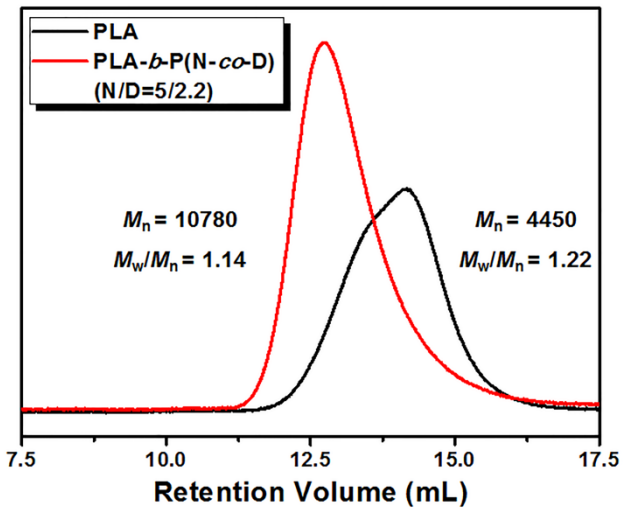

Fig. S3. GPC traces of PLA and PLA-*b*-poly(N-*co*-D) with N/D ratio of 5/2.2.

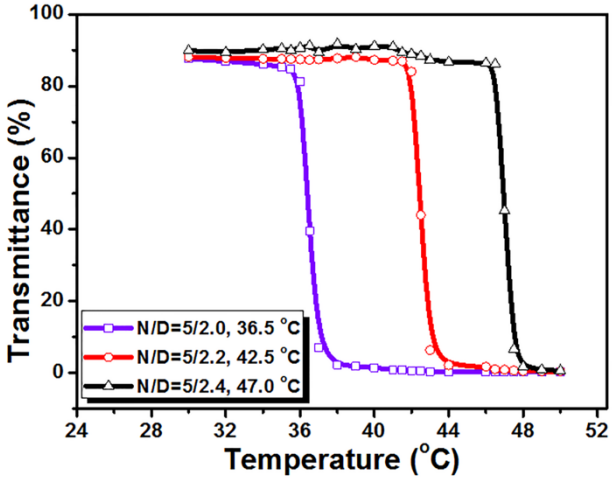

Fig. S4. The temperature-dependent transmittance curves of PLA-*b*-poly(N-*co*-D) with different N/D ratios, showing LCST of those thermosensitive copolymer at 36.5 °C, 42.5 °C and 47 °C corresponding to their N/D ratio as 5/2.0, 5/2.2 and 5/2.4.

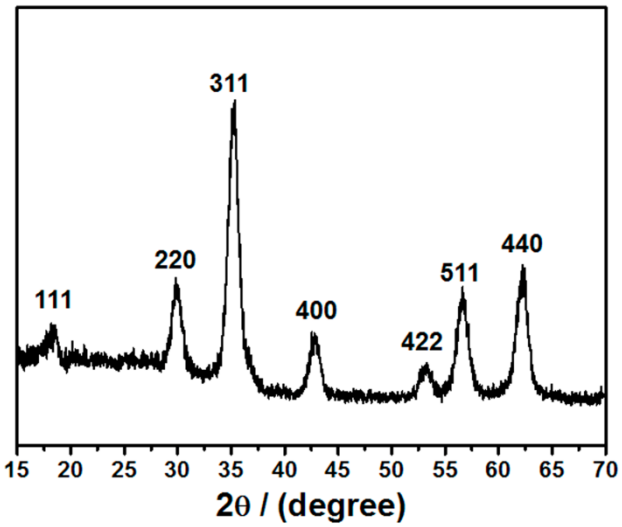

Fig. S5. The XRD result of $Mn_{0.6}Zn_{0.4}Fe_2O_4$ (MZF), showing peaks at 18.5°, 30.2°, 35.6°, 43.0°, 53.4°, 57.1° and 62.5° corresponding to the crystal plane of spinel ferrite (111), (220), (311), (400), (422), (511), and (440).

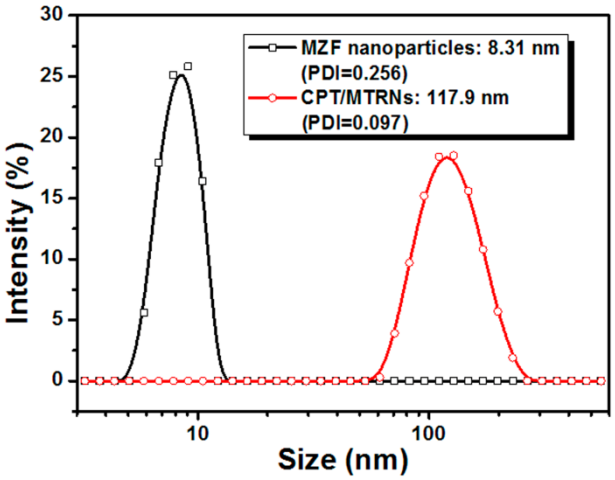

Fig. S6. The diameter distributions of MZF nanoparticles and CPT/MTRNs by DLS (Dynamic Light Scattering), coinciding with their counterparts' TEM results.

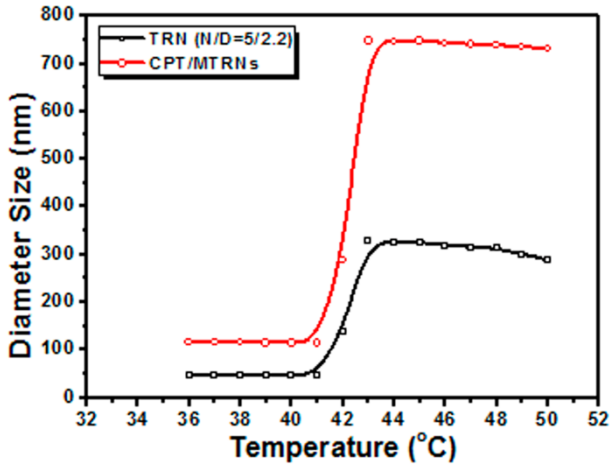

Fig. S7. The temperature-dependent diameter variation of TRN (N/D=5/2.2) and CPT/MTRNs, showing a drastic phase transition from soluble micelles (small particle) to insoluble precipitate (large aggregation).

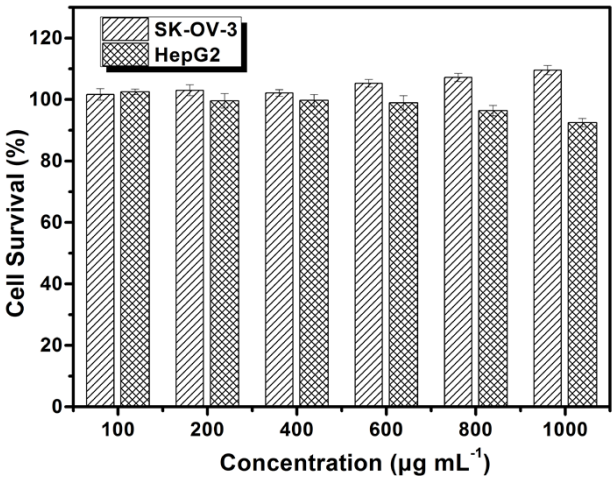

Fig. S8. The cytotoxicity of pure MTRNs on SK-OV-3 and HepG2, showing their good biocompatibility.

Reference:

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